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Osteoprotegerin (OPG) pathways in bone diseases and its application in therapeutic perspectives

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ABSTRACT
Remodeling process results from the action of the tissue-forming osteoblasts and the tissue-resorbing osteoclasts which work together in certain cell units called basic multicellular units (BMUs). The local regulation of the bone cell function includes the receptor activator of nuclear factor κB ligand (RANKL)/ receptor activator of nuclear factor κB (RANK) / Osteoprotegerin (OPG) system. These molecules are key factors linking bone formation to resorption during the bone remodeling process. The OPG/RANKL/RANK system has a role in the pathogenesis of bone loss by modulating RANK-induced osteoclastogenesis. This role has provided the rationale for durg development that treat the bone diseases. Systemic OPG therapy was used for bone disease treatment parenteral. According to our knowledge that got from researches, there is no study has been done to explore the use of the local delivery system of OPG in the bone resorption treatment. This approach may have similar osteogenic potential in bone defects that might be capable of treatment the bone loss.

Keywords: *Osteoprotegerin, bone remodeling, bone diseases.*

1. INTRODUCTION
The discovery of nuclear factor κB ligand (RANKL)/ RANK/OPG signaling pathway that has a role in the pathogenesis of bone resorption by modulating RANK-induced osteoclastogenesis have provided the rationale for the development of drugs to treat bone diseases [1].

OPG is a secretory glycoprotein of the tumour necrosis factor (TNF) receptor and plays an important role in the contol of bone resorption [2]. OPG has one ligand namely RANKL. RANKL is a Type 2 transmembrane glycoprotein that belongs to the TNF family of cytokines. RANKL is showed on the surface of osteoblasts, stromal cells, and T cells. The bound between RANKL and RANK stimulates differentiation of monocyte/macrophage progenitor cells into active and matured osteoclasts via numerous signaling pathways [3].

The role of OPG in the pathological aspects of bone diseases, such as osteoporosis associated with estrogen deficiency and periodontal disease, has been well established. Since the discovery of OPG as an inhibitor of osteoclast activity and maturation, it has opened up a new research exploration using OPG as a potential therapeutic agent for the treatment of bone diseases. Here, we reviewed the pathways of OPG in bone diseases and its application in therapeutic perspectives.

2. OSTEOPROTEGERIN (OPG)
OPG has been identified by Simonet and co-workers [4] as a secretory glycoprotein form of the superfamily of tumor necrosis factor (TNF) receptors. It inhibits osteoclast maturation and protects the physiological process of osteoclast in bone remodeling and ovariectomy-associated bone resorption.

The name osteoprotegerin derived from Latin os for bone and protegere to protect [5]. It is also named as TNF receptor-related molecule-1 (TR-1), tumor necrosis factor receptor superfamily member 11B (TNFRSF11B), osteoclastogenesis inhibitory factor (OCIF) and follicular dendritic cell receptor-(1FDCR-1).

OPG mRNA is expressed in bone, skin, liver, lung, stomach, placenta, brain and the range of other tissues. The site(s) at which it exerts its biological function is unnecessarily prdictable by the site of its expression. The binding of RANKL to RANK exerts its biological function as OPG is a secreted protein [4].

important for such growth factors as a basic fibroblast growth factor to work.

OPG has no transmembrane and cytoplasmic domain as most of the TNF superfamily receptors and is secreted as soluble protein. OPG is synthesized by osteoblast as a propeptide from which the signal peptide (with 21 amino acids) separates, generating a 380-amino acid matures peptide. It has two terminals, N and C terminus and has seven major domains (domains 1–7).

At the N-terminus of OPG is death domains (D1–D4) which are cysteine-rich structures with a characteristic of the extracellular domains of the TNFR family proteins, these domains are essential for biological activity. At the C-terminus, it has two death domains (D5-D6) that share structure features with the death domains of the TNF receptor p55, Fas and TNF-related apoptosis-inducing ligand (TRAIL) receptor, all mediate apoptosis signals. Furthermore, C- terminus has domain 7 which has the heparin binding site. Binding to heparin or heparin-like molecules is

Despite it does not correlate with the biological activity, but changes in heparin-binding with proteins affect stability, the

rate of clearance, and target cell specificity. Domain 7 is also responsible for the dimerization of OPG by disulfide bond and the dimer TNF protein is a more potent inhibitor [4-7].

OPG exists as homodimeric and monomeric forms. Both of these forms have similar potency in osteoclastogenesis inhibtion in vitro as reported by Tomoyasu and co-workers [8]. But in another study, dimeric form of OPG is a more potent RANKL inhibitor than the monomeric form, this study was reported in Schneeweis and co-workers [9].

OPG production is imitated by 1α, 25-dihydroxyvitamin D3, estrogens, pro-inflammatory cytokines such as interleukin-1 (IL-1) and TNF-α as well as transforming growth factor-β (TGF-β). OPG production is suppressed by parathyroid hormone and glucocorticoids [10, 11].

3. BIOLOGICAL FUNCTIONS OF OPG

OPG has almost one ligand, RANKL. RANKL is a type 2 transmembrane glycoprotein that belongs to the TNF family of cytokines. It is expressed on the surface of osteoblasts, stromal cells and T cells [12]. RANKL can be expressed in two forms: as a membrane anchored form and as a soluble form. These two forms can work as potential ligands that can interact with RANK and/or OPG receptors with the same biological activity [13].

In bone, RANKL specifically bind the RANK receptors, which are a type I transmembrane protein of the TNF receptor superfamily [14]. RANK receptor expressed on osteoclasts, the surface of hematopoietic osteoclast progenitors, mammary gland epithelial cells, chondrocytes, mature T cells, trophoblast cells, dendritic cells, and hematopoietic precursors [15-17].

The RANK/RANKL binding stimulates the differentiation of the monocyte/macrophage progenitor cells to osteoclasts through the activation of numerous signaling pathways involved in the osteoclast differentiation, activation and survival [2, 3].

As the binding of RANKL to RANK on pre-osteoclasts and osteoclasts is essential for their maturity and activity, OPG deters binding of RANKL to RANK. Subsequently, this binding inhibits the activation of osteoclast and osteoclastogensis and bone resorption.

Moreover, OPG also plays a key role in cell survival by its interactivity with TNF-related apoptosis inducing ligand (TRAIL). The binding of the OPG to the TRAIL and RANKL has similar affinities. As a result of the TRAIL/OPG interaction, TRAIL-induced apoptosis in several types of cells and many cancer cells is inhibited [18]. This could explain the reason for the phenomena of stimulation OPG production by tumor cells [19]. Pritzker and co-workers [20] considered the OPG as a survival factor for human microvascular endothelial cell survival as it could bind and block TRAIL-induced apoptosis.

4. ROLE OF OPG IN THE BONE DISEASE PATHOGENESIS

Various studies suggest that different bone diseases are related to alterations in OPG/RANKL/RANK system. Here we summarize several bone diseases associated with OPG/RANKL/RANK abnormalities that are estrogen secretion deficiency associated with Osteoporosis, drug-induced osteoporosis, hyperparathyroidism, Paget's disease, chronic inflammatory diseases, autoimmune diseases, osteopetrosis, bone tumors and metastases and cushing syndrome.

4.1. Estrogen secretion deficiency associated with osteoporosis.

Estrogens and androgens have direct effects on osteoclasts and inhibit bone resorption in vitro as well as inhibit the production of the pro-resorptive cytokine, interleukin (IL)-6 [21, 22]. The estrogen has a major role in the bone antiresorbing activity by stimulating OPG expression in osteoblasts. Estrogens induce the expression of the OPG gene in vitro and stimulate the OPG production from osteoblasts and inhibit the RANKL production [23]. The decrease of the estrogen secretion associated with decreasing the ovarian function leads to the decrease of the OPG production in the source cells and subsequently increase bone resorption [24].

4.2. Drug-induced osteoporosis.

The most frequent secondary form of osteoporosis is Glucocorticoid-induced osteoporosis. Glucocorticoids down-regulate OPG expression and increase the RANKL expression [25]. After glucocorticoid exposure, the rise in the osteoblastic RANKL/OPG ratio is associated with enhanced osteoclastogenesis [26].

Immunosuppressants such as cyclosporine A, rapamycin, and tacrolimus [27-29] have also been involved in the pathogenesis of post-transplant osteoporosis. These agents have been presented to significantly decrease OPG mRNA levels and protein secretion by osteoblast precursor cells in a dose-dependent fashion. It also stimulates RANKL mRNA levels in marrow stromal cells, thus substantially increasing the RANKL/OPG ratio and induce osteoclastogenesis [29]. OPG serum levels were positively correlated with serum levels of osteocalcin, parathyroid hormone, and creatinine. After renal transplantation, patients receiving cyclosporine and glucocorticoids, serum levels of OPG decreased significantly after 2 weeks from receiving drugs compared to baseline levels while creatinine clearance increased [30, 31].

4.3. Hyperparathyroidism (PTH).

Parathyroid hormone stimulates RANKL expression and inhibits OPG production by osteoblasts, and thus promotes osteoclastogenesis [32].

The effect of PTH on the production of OPG is controversial. PTH decreases OPG secretion by the osteoblast in most in vitro studies [33, 34]. However, in vivo observations are conflicting, serum OPG is not decreased in patients with hyperparathyroidism, not changed pre- or postoperatively [35]. On the other side, treatment of rats with PTH decreased OPG mRNA levels in rat femur metaphyseal and diaphyseal bone [31]. One more study showed that hyperparathyroidism is accompanied by a high serum concentration of OPG and RANKL as well as a low OPG/RANKL ratio. Both treatments with alendronate or parathyroidectomy reduce bone resorption, and increase the OPG / RANKL ratio [36, 37].

A new standard of osteoclast regulation is being developing based on the different effects of PTH on RANKL and OPG at different stages of osteoblast development. Osteoclastogenic

activity of PTH takes place primarily by suppression of OPG gene expression in first osteoblasts and raising of RANKL gene expression in mature osteoblasts [38].

4.4. Paget's disease.

The genetic polymorphisms of the OPG gene (TNFRSF11B) and RANK gene (TNFRSF11A) have been associated with Paget's disease of bone. They contribute to increasing the risk for developing the Paget's disease which is characterized by increased bone resorption by osteoclasts and uncontrolled bone remodeling [39].

Juvenile Paget's disease is a rare, autosomal recessive bone disease identified by greatly accelerated bone turnover that presents in infancy or childhood. Juvenile Paget's disease can result from osteoprotegerin deficiency caused by homozygous deletion the gene that encodes osteoprotegerin [40, 41].

4.5. Chronic inflammatory diseases.

The T cells seem to be the link between the bone resorption and inflammation. RANKL expression occurs at the surface of activated, but not quiescent, murine T cells. RANKL derived from T cell has as a signal included in optimal T cell activation. Also, T cell derived RANKL can regulate the osteoclast development and activation resulting in bone resorption so it plays a major role in the bone remodeling [42]. RANKL expression is upregulated by many soluble factors affecting bone loss, including the proinflammatory cytokines and interleukin-1 and TNF-α. T cells presents a cell-surface membrane-bound RANKL which is splited by metalloproteinases into a soluble form. Possibly, there are some functional differences between membrane-bound and soluble RANKL, with cell-bound OPGL being more functional mediators of osteoclastogenesis when measured by in vitro assays [42, 43]. Proinflammatory cytokines such as IL1 and IL6 and TNF- α increase the production of the RANKL and decrease the OPG production. These mediators and change of OPG production are associated with the chronic local inflammation and viral infections. [44, 45]. OPG act as a decoy receptor for RANKL, competing with RANK for binding with RANKL, effectively inhibiting osteoclastogenesis. Therefor, the evaluation of RANKL levels with OPG levels must accompanied as the balance of both will determine whether osteoblastic or osteoclastic activity dominates [44, 46].

4.6. Periodontitis.

In the normal periodontium, OPG is expressed by cultured human gingival fibroblasts, periodontal ligament cells and dental pulp cells, but not epithelial cells [45]. OPG is continually released by resident periodontal fibroblasts and potentially endothelial cells [47].

Macrophages and T lymphocytes that infiltrate the gingival connective tissue in periodontitis release inflammatory mediators for example, IL-1, IL-6, TNF-α and prostaglandin E2. These mediators stimulate osteoblasts to produce RANKL and induce bone resorption indirectly. T lymphocytes can also promote alveolar bone resorption by direct production of RANKL [48].

The various forms of periodontitis are differentially regulated by the expression of RANKL and OPG and it acts as indicative of disease occurrence [49].

Crotti and co-workers [50] compared the RANKL and OPG expression in the granulomatous tissue adjacent to the areas of alveolar bone loss from periodontitis patients to the tissue without periodontitis. Their results revealed the significantly higher levels of RANKLprotein in the periodontitis tissue.

Mogi et al. [51] and Bostanci et al [49] studied the alteration of RANKL and OPG levels in gingival crevice fluid (GCF) of patients with periodontitis. They found that an increase of RANKL/OPG in GCF from periodontitis patients.

In the same line Koide and coworkers [52] reported that the alveolar bone loss associated with OPG-deficient mice may be prevented by OPG which is produced locally.

4.7. Periapical disease.

OPG and RANKL have been identified in odontoblasts, ameloblasts, pulp cell lines, and periodontal ligament cells, and their expression is considered to play a role in osteoclastogenesis and bone resorption [53, 54]. In periapical disease, abnormal RANKL and OPG expression are detected, RANKL level is increased, OPG level is decreased significantly which associated with the bone absorption. The bone absorption in the periapical cyst is more active than in periapical granuloma [55].

4.8. Autoimmune diseases.

The RANKL/RANK/OPG system plays as an important link between the immune system and bone metabolism. The functioning of the OPG/RANKL system is identical to that of the interleukin–cytokine system [56].

Rheumatoid arthritis is a chronic disease that is defined by progressive synovial inflammation and joint destruction. The RANK/RANKL/OPG system plays a significant part in the pathogenesis of local and generalized bone loss in rheumatoid arthritis [57]. The synovial fibroblasts, osteoblastic stromal cells, and activated T-cells express the RANKL lead to increase the RANKL/OPG ratio,that results in enhancing osteoclastogenic in erosive arthritis [58, 59]. Otherwise, some studies have shown that serum levels of OPG and RANKL in patients with rheumatoid arthritis are higher than those in healthy people. These can be explained by anti-TNF therapy, whereby regulates the OPG/RANKL balance by stimulating the bone erosion in arthritis [59, 60].

4.9. Osteopetrosis.

Osteopetrosis is a rare inherited disorder whereby bone mass increase as a result of decreasing the osteoclastogenesis and bone resorption [61]. OPG, RANKL and their signaling pathway may play a major role in the pathogenesis of osteopetrosis. The inhibition effect of OPG on osteoclastogenesis was approved in vitro and in vivo [4].

4.10. Bone tumors and metastases.

In bone metastases, the RANK / RANKL pathway is essential in the pathology of bone destruction. Bone tumor cells can activate the osteoclasts by causing increased RANKL levels and/or decreasing OPG levels locally, resulting in excessive osteoclast activity [62, 63]. In severe osteolysis, RANKL/OPG ratio is increased and involved in the development of benign and malignant bone tumors and the progress of osteolytic lesions by tumor metastases [64, 65]. RANKL expression is increased in bone metastases associated with different types of solid tumors, including breast cancer while the expression of OPG is inhibited. These changes caused by secretion the factors such as IL-1, IL-11 and PGE2 by tumor cells present in the bone marrow [66, 67].

4.11. Cushing syndrome.

Cushing syndrome defined as the signs and symptoms associated with prolonged exposure to incorrectly high levels of the hormone cortisol. This can result from diseases that caused the excess cortisol, adrenocorticotropic hormone levels, taking glucocorticoid drugs [68].

Serum OPG levels also positively correlated with morning serum cortisol. In patients with Cushing syndrome, serum OPG levels are higher than healthy control subjects, but it remained unchanged after the recovery, even the bone is restored. The elevation of OPG level could be due to persistent damage to the cardiovascular system by glucocorticoid [69-71].

5. OVERVIEW OF THERAPEUTIC PERSPECTIVES OF OPG IN BONE DISEASES

As the OPG has a key role in the bone diseases, there are various experimental and clinical trials on the use of OPG in treatment of different bone diseases. It was previously reported that OPG-chitosan matrices and gel increase the in vitro proliferation of normal human osteoblasts and fibroblasts cells that used later to treat the critical size defects on parietal bone of rabbit. The results of this studies showed that bioresorbable OPG-chitosan material induced the formation of a significant quantity of bone in a critical-sized parietal bone defect in a rabbit model [72-74].

Simonet et al. [4] and Yasuda et al. [6] reported that the increasing in bone mass in mice after treatment with OPG at 10 mg/kg/day for 7 days and 24 mg/ kg/day for 14 days. Furthermore, OPG treatment at a dose of 5 mg/ kg/day for 14 days has been completely prevented ovariectomy-induced bone loss in rats [4]. There is another study confirmed the hypocalcemia effects of OPG after injection in normal rats. The result showed that increases of density of bone mineral and volume of bone accompanied by a decrease of osteoclast number. The facts suggested that the reduction of the calcium concentration in serum of normal rats by administration of OPG is due to suppression of bone resorption by inhibition of osteoclastogenesis, especially maturation of osteoclasts [8].

Bolon and co-workers transfer OPG gene mediated vehicle by a single injection of a recombinant adenovirus carrying the OPG-Fc gene, prevents bone density loss induced by ovariectomy period [75]. In addition, a single intravenous injection of OPG in young growing rats leads to a significant increase in bone volume and density, which are associated with suppression of osteoclastic bone resorption [76].

Other preclinical studies demonstrated a potential therapeutic role of OPG in the prevention and reduction of lytic bone lesion associated with skeletal tumor. Lamoureux et al. [77] suggested the targeting of RANKL may has the potential to modify the osteosarcoma and suppress its development.

In experimental periodontitis, human OPG has strong preventive effects on alveolar bone loss in rats [78]. Yao et al. [79] revealed that OPG with BMP-2 improves osteoblastogenesis and new bone formation by recruitment of mesenchymal stem cells [79]

OPG treatment is used in preclinical bone metastasis models. The result revealed that the proliferation of tumor cell is decreased and apoptosis of tumor cell osteoclast activity is decreased. Whereby the bone turnover and the pro-tumor growth factors releasing from the bone matrix are prevented [64].

Additionally, there are a few clinical trials on the use of OPG systemically. One of these studies is done by using a single subcutaneous dose of human recombinant OPG (3 mg/kg) in post-menopausal women [80, 81]. It was stated that biochemical markers of bone resorption have reduced and OPG can reduce bone turnover.

Another study performed by Body et al. [82], who used recombinant OPG in patients with bone disease related to breast carcinoma or multiple myeloma. The results indicated that recombinant OPG is effective in reducing bone resorption marker levels as an accepted treatment for these diseases.

6. CONCLUSIONS

Bone resorption-related diseases, such as periodontitis, osteoporosis or rheumatoid arthritis, affect many people worldwide. New doors have been opened for bone research and drug development. The different studies illuminate the fact of that OPG regulates osteoclastogenesis via the RANKL-OPG pathway. Thus, enhancing OPG production may be accompanied with an increase in bone mass.

Since the discovery of OPG as an inhibitor of osteoclast activity and maturation, it has opened up a new research exploration using OPG as a potential therapeutic agent for the treatment of bone diseases. Up to our knowledge, there is a few research carried out to explore the use of local OPG therapy in the treatment of bone resorption. As a critical therapeutic concern, it is to target specifically the bone tissue to avoid prospective undesired effects on other tissues. Systemic therapy may lead to unwanted side effects, therefore local therapy of OPG may be efficient as an alternative therapy.

Despite the interest of the various studies targeting the OPG pathways in skeletal diseases, further preclinical and clinical studies are needed. One possible approach is the development of local delivery of OPG that may have similar potential in treatment of bone loss.

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8. ACKNOWLEDGEMENTS

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